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<p>(21) International Application Number: PCT/US99/04009</p> <p>(22) International Filing Date: 24 February 1999 (24.02.99)</p> <p>(30) Priority Data: 09/027,649 24 February 1998 (24.02.98) US</p> <p>(71) Applicant (for all designated States except US): UNIVERSITY OF VIRGINIA [US/US]; The Rector and Visitors of the University of Virginia, Washington Hall, East Range, Charlottesville, VA 22903-2442 (US).</p> <p>(72) Inventor; and (75) Inventor/Applicant (for US only): LINDEN, Joel, M. [US/US]; 207 Harvest Drive, Charlottesville, VA 22903 (US).</p> <p>(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).</p>		<p>(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>
<p>(54) Title: ANTAGONISTS OF A2B HUMAN ADENOSINE RECEPTORS</p> <p>(57) Abstract</p> <p>The invention concerns the use of 8-phenylxanthines, 8-cycloalkylxanthines or 8-substituted xanthine derivatives to specifically modulate the physiologic role of the A2B adenosine receptor.</p> <div data-bbox="941 1050 1396 1806"><p>Figure A: Time course of $[Ca^{2+}]_i$ response to NECA and IB-MECA in HMC-1 cells. The solid line represents 10 μM NECA, showing a sharp increase in $[Ca^{2+}]_i$ from approximately 250 nM to 700 nM. The dotted line represents 10 μM IB-MECA, showing a much smaller response.</p><p>Figure B: Dose-response curves for NECA (circles) and IB-MECA (squares) in HMC-1 cells. The x-axis is [ANTAGONIST], LOG M, ranging from -12 to -4. The y-axis is CYCLIC AMP (pmol/ml), ranging from 0 to 70. NECA (circles) shows a higher EC₅₀ (approximately 10^{-7} M) compared to IB-MECA (squares, approximately 10^{-8} M).</p></div>		

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ANTAGONISTS OF A2B HUMAN ADENOSINE RECEPTORS

BACKGROUND OF THE INVENTION**1. Field of the Invention**

The present invention relates to methods for the treatment or prevention of disease states induced by activation of the A2B receptor and mast cell activation.

2. Description of the Related Art

A key early event in allergic responses is the activation of mast cells by allergens. For example, in asthma, exposure to an allergen such as ragweed, triggers the release of allergic mediators such as histamine, leukotrienes, etc. from mast cells.

The action of allergens to trigger mast cell degranulation is enhanced by adenosine in asthmatics, but not in non-asthmatics (Bjorck T, Gustafsson LE, Dahlen SE: Isolated bronchi from asthmatics are hyper responsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. *Am.Rev.Respir.Dis.* 1992;145:1087-1091). Theophylline is a xanthine that is known to block adenosine receptors and is effective therapeutically to treat asthma (Barnes PJ, Pauwels RA: Theophylline in the management of asthma: time for reappraisal?. *European.Respiratory.Journal.* 1994;7:579-591). For this reason, theophylline is thought to ameliorate the symptoms of asthma, at least in part by blocking adenosine receptors. However, enprofylline, another xanthine that also is used to treat asthma in Europe, was found not to block adenosine receptors in the therapeutic concentration range of 20-50 uM (Chapman KR, Ljungholm K, Kallen A: Long-term xanthine therapy of asthma. Enprofylline and theophylline compared. International Enprofylline Study Group. *Chest* 1994;106:1407-1413). Hence it was concluded that enprofylline does not work by blocking adenosine receptors. However, this conclusion was based on an examination of enprofylline binding only to two of the four known adenosine receptor subtypes, A1 and A2A receptors.

Applicant and others have recently discovered that the A₃ adenosine receptor on mast cells are responsible for adenosine-stimulated release of allergic mediators in rodent species. (Jin X, Shepherd RK, Duling BR, Linden J: Inosine binds to A₃ adenosine receptors and stimulates mast cell degranulation. *J.Clin.Invest.* 1997;100:2849-2857; Ramkumar V, Stiles GL, Beaven MA, Ali H: The A₃ adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J.Biol.Chem.* 1993;268:16887-16890). These findings are misleading in that applicant has found that the A₃ receptor is not involved in the release of allergic mediators from other species, including human and dog. Rather, applicant discovered that in canine and human mast cells the A_{2B} and not the A₃ adenosine receptor is responsible for adenosine-facilitated mast cell degranulation (Auchampach JA, Jin J, Wan TC, Caughey GH, Linden J: Canine mast cell adenosine receptors: cloning and expression of the A₃ receptors and evidence that degranulation is mediated by the A_{2B} receptor. *Mol.Pharmacol.* 1997;52:846-860). Fig. 1 of the instant application shows that NECA (a nonselective agonist that activates A_{2B} and A₃ receptors) causes intracellular Ca²⁺ and cyclic AMP accumulation in the human mast cell line, HMC-1. IB-MECA, a potent and selective agonist of the A₃ receptor is poorly effective. These data suggest that the A_{2B} receptor mediates these responses in HMC-1 human mast cells. Another published report also suggests that activation of A_{2B} receptors is responsible for triggering interleukin-8 release from human HMC-1 mast cells (Feoktistov I, Biaggioni I: Adenosine A_{2B} receptors evoke interleukin-8 secretion in human mast cells - An enprofylline-sensitive mechanism with implications for asthma. *J.Clin.Invest.* 1995;96:1979-1986).

8-Phenylxanthines, methods of their synthesis and their use in human and veterinary therapy for conditions associated with the cell surface effects of adenosine have been described (EP 0 203 721, published 12/3/86). However, this publication is silent as to whether adenosine receptors mediate this response and if so, which adenosine receptor subtype. Also, the subtype specificity of disclosed compounds is not described. In WO 90/00056, a group of 1,3-

unsymmetrical straight chain alkyl-substituted 8-phenylxanthines were described as being potent bronchodilators. This disclosure is likewise silent as to the role of adenosine and the subtype specificity of disclosed compounds.

5 Methods of treating conditions related to the physiological action of adenosine have, to date, proven inferior due to the presence of multiple subtypes present in the animal tissue utilized (R.F. Bruns *et al.*, (1986) *Mol. Pharm.* 29:331-346) and the differences between species in the affinity for adenosine analogs and the physiological effects of adenosine (Ukera *et al.*, (1986) *FEBS Lett*, 209:122-128).

10 SUMMARY OF THE INVENTION

The present invention concerns the use of compounds identified as specific modulators of adenosine's physiological actions. The pharmacology of these compounds is characterized through the use of cloned human adenosine receptors of the A1, A2A, A2B and A3 class and their subtypes. Applicant has
15 found that compounds identified as antagonists of the A2B adenosine receptor subtype are useful in preventing mast cell degranulation and are therefore useful in the treatment or prevention of disease states induced by activation of the A2B receptor and mast cell activation. These disease states include but are not limited to asthma, myocardial reperfusion injury, allergic reactions including but
20 not limited to rhinitis, poison ivy induced responses, urticaria, scleroderm arthritis, other autoimmune diseases and inflammatory bowel diseases. The present invention is based on the finding that antagonists of the A2B adenosine receptor subtype have anti-inflammatory action.

25 Through the use of homogenous, recombinant adenosine receptors, the identification and evaluation of compounds which have selectivity for a single

receptor subtype have now been accomplished. Moreover, because of the variable effects of adenosine documented in other species, the utilization of human adenosine receptor subtypes is advantageous for the development of human therapeutic adenosine receptor agonists, antagonists or enhancers. In previous research conducted by the Applicant, compounds which unexpectedly exhibit selective binding affinity for the human A2B adenosine receptor were identified, along with methods for using such compounds to overcome the disadvantages of using compounds of uncharacterized specificity. The compounds specifically block activities mediated through the activation of the A2B receptor subtype without substantially blocking the activities of other adenosine receptor subtypes. In particular, Applicant found that the use of such compounds, identified through the use of recombinant human adenosine receptors A1, A2A, A2B and A3, and functional assays, can specifically modulate the physiologic role of adenosine activation of various receptors.

Applicant has developed for the first time a radioligand binding assay for the A2B adenosine receptor (Linden, J. et al. US patent application serial no 08/670,175, filed June 20, 1996, the entire disclosure of which is herein incorporated by reference). Using this assays system, applicant has discovered that enprofylline, in the therapeutic concentration range of 20-50 μ M used to treat asthma, blocks recombinant human A2B adenosine receptors, but is a much weaker antagonist of other adenosine receptor subtypes (Figure 2 and Table 1).

The release of enzymes, bioactive amines and arachidonic acid metabolites following mast cell activation causes vasoconstriction, edema, leukocyte accumulation, and ultimately, tissue damage. Mast cell degranulation is a component of myocardian reperfusion injury, hypersensitivity reactions

(asthma, allergic rhinitis, and urticaria), ischemic bowel disease, autoimmune inflammation, and atopic dermatitis. Highly specific A2B adenosine receptor antagonists can be used to treat or prevent these diseases and pathologic effects that result from mast cell degranulation.

5 Mast cell degranulation is clearly involved in the pathophysiology of allergies such as asthma. Autoimmune diseases are also characterized by immune reactions which attack targets, including self-proteins in the body such as collagen, mistaking them for invading antigens. The resulting damage, caused at least in part by mast cell degranulation, is amenable to treatment by the method of this invention which comprises administration of selective A2B
10 adenosine receptor antagonists effective to inhibit mast cell degranulation. Among these types of diseases, all of the following, but not limited to these, are amenable to treatment by the administration of selective A2B adenosine receptor antagonists: Addison's disease (adrenal), autoimmune hemolytic anemia (red
15 cells), Crohn's disease (gut), Goodpasture's syndrome (kidney and lungs), Grave's disease (thyroid), Hashimoto's thyroiditis (thyroid), idiopathic thrombocytopenic purpura (platelets), Insulin-dependent diabetes militus (pancreatic beta cells), multiple sclerosis (brain and spinal cord), myasthenia gravis (nerve/muscle synapses), Pemphigus vulgaris (skin), pernicious anemia
20 (gastric parietal cells), poststreptococcal glomerulonephritis (kidney), psoriasis (skin), rheumatoid arthritis (connective tissue), sclerodenna (heart, lung, gut, kidney), Sjogren's syndrome (liver, kidney, brain, thyroid, salivary gland), spontaneous, infertility (sperm), systemic lupus erythematosus (DNA, platelets, other tissues).

25

Disease states associated with A2B adenosine receptor activation and mast cell degranulation include, but are not limited to asthma, myocardial

reperfusion injury, allergic reactions including but not limited to rhinitis, asthma, poison ivy induced responses, urticaria, scleroderma, arthritis, and inflammatory bowel diseases.

5 The present invention is directed to the discovery that antagonists of A2B receptors are anti-inflammatory in man. A3 adenosine receptors also have an anti-inflammatory action, but are most important in rodent species. It has been found that enprofylline, a compound already used to treat asthma, blocks A2B adenosine receptors and that human HMC-1 mast cells have A2B receptors and that 8-phenylxanthines that block human A2B adenosine receptors are useful in
10 the treatment or prevention of disease states induced by activation of the A2B receptor and mast cell activation. Also, applicant has discovered that BW-A493 is a potent and selective antagonist of human A2B adenosine receptors (Table 1).

15 A further aspect of the invention is the treatment or prevention of asthma, bronchoconstriction, allergic potentiation, inflammation or reperfusion injury in a human by administering to the human an amount of an adenosine A2B receptor specific inhibitor comprising an 8-phenylxanthine or 8-phenylxanthine derivative effective to antagonize activation of the adenosine receptor of the A2B subtype by adenosine.

20 The invention also relates to a method for treating a human suffering from an autoimmune disease selected from the group consisting of Addison's disease (adrenal), autoimmune hemolytic anemia (red cells), Crohn's disease (gut), Goodpasture's syndrome (kidney and lungs), Grave's disease (thyroid), Hashimoto's thyroiditis (thyroid), idiopathic thrombocytopenic purpura (platelets), insulin-dependent diabetes militus (pancreatic beta cells), multiple
25 sclerosis (brain and spinal cord), myasthenia gravis (nerve/muscle synapses),

Pemphigus vulgaris (skin), pernicious anemia (gastric parietal cells), post-streptococcal glomerulonephritis (kidney), psoriasis (skin), rheumatoid arthritis' (connective tissue), scleroderma (heart, lung, gut, kidney), Sjogren's syndrome (liver, kidney, brain, thyroid, salivary gland), spontaneous infertility (sperm), and systemic lupus erythematosus (DNA, platelets, other tissues), which comprises administering to the human an effective amount of a selective A2B adenosine receptor antagonist comprising a xanthine or a xanthine derivative having a meta-substituted acidic aryl at the 8 position to inhibit mast cell degranulation.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate that 5-N-ethylcarboxamidoadenosine (NECA) but not N⁶-(2-iodo)benzyl-5'-N-methylcarboxamidoadenosine (IB-MECA) stimulates human mast cells to mobilize calcium and to accumulate cyclic AMP.

15 Figure 2A is an illustration of competitive binding studies of theophylline and enprofylline for the rhA2B adenosine receptor.

Figure 2B is an illustration of competitive binding studies of theophylline and enprofylline for the rhA3 adenosine receptor.

20 Figures 3A and 3B illustrate the functional effects of theophylline and enprofylline in modulating cAMP in HEK 293 cells transfected with A2B adenosine receptor cells.

Figure 4 is an illustration of the functional effects of theophylline and enprofylline in modulating cAMP in HEK 293 cells transfected with A3 adenosine receptor cells.

25 Figure 5 is an illustration of the effects of theophylline and enprofylline on inositol-(1,4,5)-trisphosphate (IP₃) generation.

Figure 6 is an illustration of the effects of theophylline and enprofylline on intracellular calcium mobilization.

Figure 7 is an illustration of structures to further identify compounds described in this application.

5 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to a method for achieving a blockade of the mast cell degradation response induced through adenosine activation of the A2B adenosine receptor subtype. The method comprises contacting cells bearing the A2B receptor with an amount of an adenosine A2B receptor subtype specific inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-
10 substituted xanthine derivative effective to block activation of the receptor by adenosine.

Further, the invention relates to a method for treating or preventing myocardial ischemia, inflammation, brain arteriole diameter constriction, and/or
15 the release of allergic mediators. The method comprises using a specific inhibitor of the A2B adenosine receptor subtype to inhibit effects induced by adenosine mediated mast cell degranulation by contacting A2B receptor bearing mast cells with an amount of a selective A2B inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8- substituted xanthine derivative
20 effective to prevent mast cell degranulation.

Further the invention relates to a method for preventing or treating asthma, bronchoconstriction, allergic potentiation, inflammation or reperfusion injury in a human. The method comprises administering to the human an effective amount of an adenosine A2B receptor specific inhibitor comprising an
25 8-phenyl or 8-cycloalkyl substituted xanthine or substituted xanthine derivative

to antagonize activation of the adenosine receptor of the A2B subtype by adenosine.

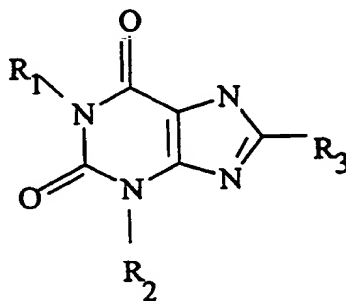
Further, the invention relates to a method for preventing mast cell degranulation in a human. The method comprises administering to the human
5 an amount of an adenosine A2B receptor specific inhibitor comprising an 8-substituted xanthine or 8- substituted xanthine derivative effective to antagonize activation of the adenosine receptor of the A2B subtype by adenosine.



Further, the invention relates to a method for treating an autoimmune disease selected from the group consisting of Addison's disease (adrenal),
10 autoimmune hemolytic anemia (red cells), Crohn's disease (gut), Goodpasture's syndrome (kidney and lungs), Grave's disease (thyroid), Hashimoto's thyroiditis (thyroid), idiopathic thrombocytopenic purpura (platelets), Insulin-dependent diabetes militus (pancreatic beta cells), multiple sclerosis (brain and spinal cord), myasthenia gravis (nerve/muscle synapses), Pemphigus vulgaris (skin),
15 pernicious anemia (gastric parietal cells), poststreptococcal glomerulonephritis (kidney), psoriasis (skin), rheumatoid arthritis' (connective tissue), scleroderma (heart, lung, gut, kidney), Sjogren's syndrome (liver, kidney, brain, thyroid, salivary gland), spontaneous infertility (sperm), and systemic lupus erythematosus (DNA, platelets, other tissues). The method comprises
20 administration to a patient in need thereof of an effective amount of a selective A2B adenosine receptor antagonist comprising an 8- substituted xanthine or 8-substituted xanthine derivative to inhibit mast cell degranulation.

Further, the invention relates to a method for the treatment or prevention of disease states mediated through activation of the A2B subtype of the
25 adenosine receptor on mast cells by prevention of mast cell degranulation

through blockade of the A2B subtype of the adenosine receptor. The method comprises contacting mast cells with an inhibitory effective amount of an adenosine A2B receptor specific inhibitor comprising an 8- substituted xanthine or 8- substituted xanthine derivative specific for the A2B receptor subtype. The disease state includes asthma, myocardial reperfusion injury, allergic reactions including but not limited to rhinitis, poison ivy induced responses, urticaria, scleroderma, arthritis, and inflammatory bowel diseases.

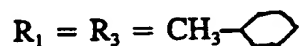
A preferred 8-phenyl or 8-cycloalkyl substituted xanthine or 8- substituted xanthine derivative has the formula:



wherein R_1 is a hydrogen, an alkyl, a cycloalkyl, or an aryl; R_2 is a cycloalkyl or an aryl; and R_3 is a phenyl, substituted phenyl, cycloalkyl or substituted cycloalkyl. Specifically, when the 8-phenyl substituted xanthine or 8-phenyl substituted xanthine derivative is BW 493, $R_1 = R_2 = -CH_2-$  and R_3 is -C=C-COO-. When enprofylline is selected as the 8-phenyl substituted xanthine or 8-phenyl substituted xanthine derivative, $R_1 = H$, $R_2 = C-C-C$ and $R_3 = H$.

Preferably the 8-phenyl or 8-cycloalkyl substituted xanthine or 8- substituted xanthine derivative has an affinity for the A2B subtype of the human adenosine receptor which is at least one order of magnitude greater than the

affinity for either the A1 or A2 subtypes of the human adenosine receptor effective to antagonize activation of the adenosine receptor of the A2B subtype by adenosine when:



5 More preferably, the 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative has a pKi for the A2B subtype of 7 or greater and a pKi for other adenosine receptor subtypes of 6 or less. Most preferably, the 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative is BW 493.

10 The following examples are provided to further define but not to limit the invention defined by the foregoing description and the claims which follow:

Example 1

Functional Responses Of Human HMC-1 Mast Cells to NECA and IB-MECA: In tests as described in "Canine Mast Cell Adenosine Receptors: Cloning and Expression of the A3 Receptor and Evidence that Degranulation is Mediated by the A2B Receptor," *Molecular Pharmacology*, 52:1-15 (1997) to Auchampach *et al.*, that reference being incorporated herein by reference, intact cells were treated with the A3-selective agonist IB-MECA and the nonselective agonist NECA.

20 Figures 1A and 1B show (A) Intracellular Ca^{2+} accumulation measured in cells pretreated with the Ca^{2+} -sensitive fluorescent reporter, FURA and (B) Cyclic AMP accumulation measured by radioimmunoassay. The results are typical of triplicate experiments. Figures 1A and 1B show that NECA, but not IB-MECA stimulates canine mast cells to mobilize calcium and to accumulate

cyclic AMP. Agonists of A1 or A2A adenosine receptors do not have these effects. These data suggest that canine mast cells are activated by A2B rather than A3 adenosine receptors.

Example 2

5 **Binding of Enprophylline and Theophylline to Human Adenosine Receptors:**
The xanthines theophylline and enprophylline (See Figure 7) are used clinically to treat asthma. However, enprophylline has been reported to bind weakly to adenosine receptors. Lunell *et al.*, Effects of enprophylline, a xanthine lacking adenosine receptor antagonism, in patients with chronic obstructive lung disease,
10 *European Journal of Clinical Pharmacology* 22:395-402 (1982).

Competition for specific radioligand binding of enprophylline and theophylline was measured on membranes prepared from cells expressing (A) recombinant human A2B adenosine receptors and (B) recombinant human A3 adenosine receptors as described in "Molecular Characterization of Recombinant
15 Human Adenosine Receptors," *Drug Development Research*, 39:243-252 (1996) to Robeva *et al.*, which is incorporated herein by reference.

As shown in Figures 2A and 2B, each point is the mean standard error of the mean (SEM) of triplicate determinations. The results are typical of three experiments. In the competition binding studies shown, theophylline and
20 enprophylline compete for [³H]1,3-diethyl-8-phenylxanthine ([³H]DPX, 5nM) binding to rhA2B adenosine receptors as shown in Figure 2A. Both antagonist have higher affinities for human A2B adenosine receptors than for human A3 adenosine receptors (see Figure 2B) with A2B K_i values of 7.1 μM and 5.6 μM for theophylline and enprophylline, respectively.

Example 3

Functional Antagonism by Enprofylline and Theophylline of Recombinant A2B Receptor-Mediated Cyclic AMP Accumulation: Agonists were used to modulate cAMP in HEK 293 cells stably transfected the rhA2B adenosine receptors or rhA3 adenosine receptors. This procedure is also described in the Auchampach *et al.* publication.

Figures 3A and 3B shows antagonists of NECA-stimulated cyclic AMP accumulation in transfected HEK-293 cells by (A) theophylline and (B) enprofylline. Line (C) is Schild analysis of the data shown in (A) and (B).

NECA or IB-MECA produced a dose-dependent functional response in cells expressing rhA2B adenosine receptors or rhA3 adenosine receptors respectively. The addition of theophylline or enprofylline produced a progressive shift to the right in the potency of NECA in these functional assays. Schild analyses of the data gave A2B K_i values of 16.7 μ M for theophylline and 17.1 μ M for enprofylline.

For rhA3 adenosine receptors, the A3-selective agonist, IB-NECA was used to generate dose-response curves for inhibition of isoproterenol-stimulated cyclic accumulation in the absence or presence of different concentrations of theophylline or enprofylline. IB-MECA produced dose-dependent inhibition of cAMP accumulation stimulated by 1 μ M isoproterenol; the maximum inhibition was 50-70%. The presence of increasing concentrations of either theophylline or enprofylline shifted the dose-response curve progressively to the right, with K_i values of 27.6 μ M for theophylline and 39.6 μ M for enprofylline based on Schild analysis (see Figure 4). For both A2B and A3 receptors, the K_i values of theophylline and enprofylline obtained from cAMP functions assays are in

good agreement with the K_i values calculated from radioligand competition binding assays.

Example 4

5 **Effect of Theophylline and Enprofylline of IP_3 generation:** On agonist stimulation, A2B adenosine receptors activate phospholipase C, leading to inositol-(1,4,5)-trisphosphate (IP_3) formation. Characteristically, A2B-mediated effects are insensitive to blockage by pertussis toxin.

10 In this example, untransfected HEK-293 (HEK 293) or cells transfected with recombinant human A2B adenosine receptors were treated with the indicated compounds. IP_3 was measured in cells pretreated with [3H]inositol. This procedure is also described in the Auchampach *et al.* publication.

As shown in Figure 5, NECA at 10 μM produced a 3.5 fold increase in IP_3 formation in rhA2B adenosine receptor transfected HEK 293 cells. At 250 μM , both theophylline and enprofylline were able to block the increase in IP_3 produced by 10 μM NECA in human A2B adenosine receptor transfected HEK 15 293 cells. Neither antagonist affected basal levels of inositol phosphates.

Example 5

20 **Effect of Theophylline and Enprofylline on the Ca^{2+} Mobilization:** The activation of the phospholipase C pathway leads to intracellular calcium mobilization. NECA produces a dose-dependent increase in intracellular Ca^{2+} content in human A2B adenosine receptor transfected cells.

HEK-293 cells transfected with recombinant human A2B adenosine receptors were treated with the compounds indicated in Figure 6. Calcium

mobilization was measured in cells preloaded with FURA. Again, this procedure is also described in the Auchampach *et al.* publication.

As shown in Figure 6, theophylline or enprofylline at 100 μ M totally blocks the Ca^{2+} response induced by 1 μ M NECA.

5

Example 6

Screening to Identify Selective Antagonists of Recombinant Human A2B Adenosine Receptors: A series of compounds was screened to identify potent A2B selective antagonists. This was done in competition binding assays using recombinant human A1, A2A, A2B or A3 adenosine receptors, similar to that illustrated in Figures 2A and 2B.

10

Table 1

The following K_i values (nM) show that BW-A493 is a potent and selective A2B antagonist:

		<u>BW-A493</u>	<u>Enprofylline</u>
15	A1	4980 ± 553	156,000 ± 109,000
	A2A	1518 ± 797	32,000 ± 7,800
	A2B	198± 52	7,000 ± 1,850
	A3	922 ± 399	65,000 ± 12,100

20

One should note that the lowest K_i value corresponds to the highest affinity; BW-A493 and enprofylline are A2B selective. As shown above, BW-A493 is approximately 35 times more potent than enprofylline as an antagonist of human A2B adenosine receptors.

Examples 1 to 5 indicate that known anti-inflammatory compounds are antagonists of A2B. The Examples establish that enprofylline, a compound used

to treat asthma, but which previously had an unknown mechanism of action, blocks human A2B adenosine receptors and that human HMC-1 mast cells have A2B receptors indicating that antagonists of A2B as well as A3 adenosine receptors have anti-inflammatory action. Example 6 identifies BW-A493 as a selective antagonist of human A2B adenosine receptors.

Enprofylline can be used in moderately severe asthmatic patients. Typically, a bolus injection of 1.5 mg/kg enprofylline is given over 20 minutes and then a maintenance infusion of 0.4 mg/kg/h is given for up to 24 hours.

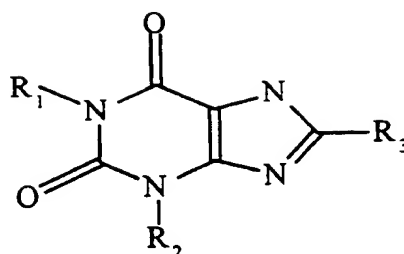
While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the variations, adaptations, modifications as come within the scope of the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. The use of an adenosine A₂B receptor subtype specific inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted
5 xanthine derivative effective to block activation of the receptor by adenoside to prepare a medicament useful for achieving blockade of inflammatory responses.
2. The use of a specific inhibitor of the A₂B adenosine receptor subtype to prepare a medicament useful for treating or preventing myocardial ischemia,
10 inflammation, brain arteriole diameter constriction, or the release of allergic mediators.
3. The use of an adenosine A₂B receptor specific inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative
15 to antagonize activation of the adenoside receptor of the A₂B subtype by adenoside to prepare a medicament useful for preventing or treating asthma, bronchoconstriction, allergic potentiation, inflammation or reperfusion injury in a human.
- 20 4. The use of an adenosine A₂B receptor specific inhibitor comprising an 8-phenol or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative effective to antagonize activation of the adenoside receptor of the A₂B subtype by adenoside to prepare a medicament useful for preventing mast cell degranulation in a human.
- 25 5. The use of an adenosine A₂B receptor specific inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative effective to reduce vasoconstriction in the vasculature without any substantial effect (binding or blockade) of the A₁ or A₂A subtypes of the adenoside receptor
30 to prepare a medicament useful for achieving blockade of vascular constriction induced through activation of the A_{2B} subtype of the adenosine receptor in a primate.

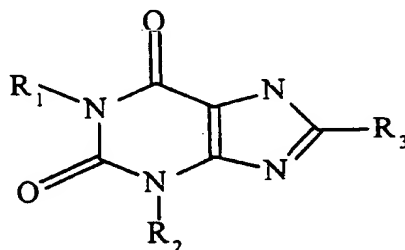
6. The use of a selective A2B adenosine receptor antagonist comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative effective to inhibit mast cell degranulation to prepare a medicament useful for treating an autoimmune disease selected from the group consisting of
- 5 Addison's disease (adrenal), autoimmune hemolytic anemia (red cells), Crohn's disease (gut), Goodpasture's syndrome (kidney and lungs), Grave's disease (thyroid), Hashimoto's thyroiditis (thyroid), idiopathic thrombocytopenic purpura (platelets), Insulin-dependent diabetes melitis (pancreatic beta cells), multiple sclerosis (brain and spinal cord), myasthenia gravis (nerve/muscle
- 10 synapses), Pemphigus vulgaris (skin), pernicious anemia (gastric parietal cells), poststreptococcal glomerulonephritis (kidney), psoriasis (skin), rheumatoid arthritis (connective tissue), scleroderma (heart, lung, gut, kidney), Sjogren's syndrome (liver, kidney, brain, thyroid, salivary gland), spontaneous infertility (sperm), and systemic lupus erythematosus (DNA, platelets, other tissues).
- 15
7. The use of an adenosine A2B receptor specific inhibitor comprising an 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative specific for the A2B receptor subtype to prepare a medicament useful for the treatment or prevention of disease states mediated through activation of the A2B
- 20 subtype of the adenosine receptor on mast cells by prevention of mast cell degranulation through blockade of the A2B subtype of the adenosine receptor.
8. The use of claim 7 wherein the disease state associated with A2B adenosine receptor activation and mast cell degranulation includes asthma,
- 25 myocardial reperfusion injury, allergic reactions including but not limited to rhinitis, poison ivy induced responses, urticaria, scleroderma, arthritis, and inflammatory bowel diseases.
9. The use of any one of claims 1-8, wherein the xanthine derivative is
- 30 enprofylline.
10. The use of any one of claims 1-8 wherein the 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative has the formula:

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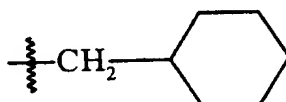


wherein the R_1 is hydrogen, alkyl, cycloalkyl or aryl; R_2 is cycloalkyl or aryl; and R_3 is phenyl, cycloalkyl, substituted phenyl, or substituted cycloalkyl.

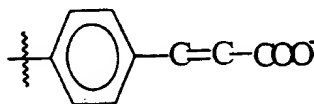
11. The use of any one of claims 1-8 wherein the 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative has the formula:



wherein $R_1 = R_2 =$



and R_3 is



12. The use of any one of claims 1-8 wherein the 8-phenyl or 8-cycloalkyl substituted xanthine or 8-substituted xanthine derivative has an affinity for the A2B subtype of the human adenosine receptor which is at least one order of magnitude greater than the affinity for either the A1 or A2A subtypes of the human adenosine receptor effective to antagonize activation of the adenosine receptor of the A2B subtype by adenosine.

13. The use of any one of claims 1-8, wherein the 8-phenyl or 8-cycloalkyl substituted xanthine or 8- substituted xanthine derivative has the pKi for the A2B subtype of 7 or greater and a pKi for other adenosine receptor subtypes of 6 or less.

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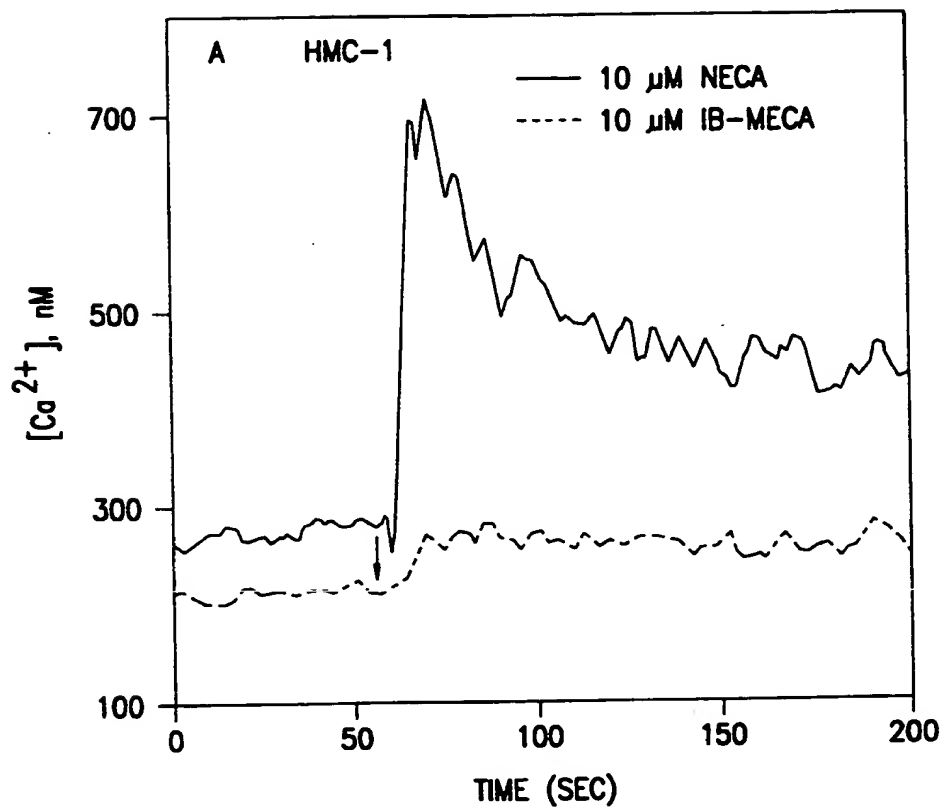


FIG. 1A

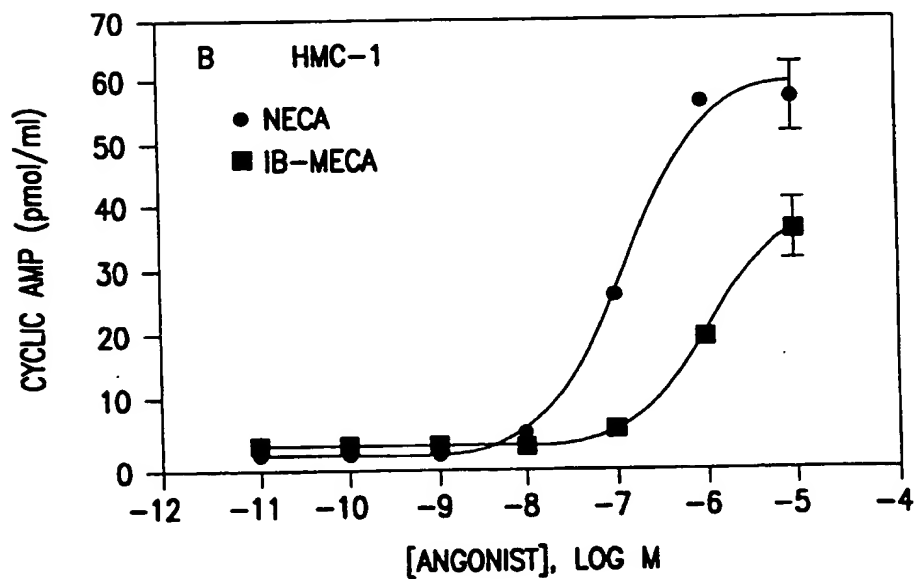


FIG. 1B

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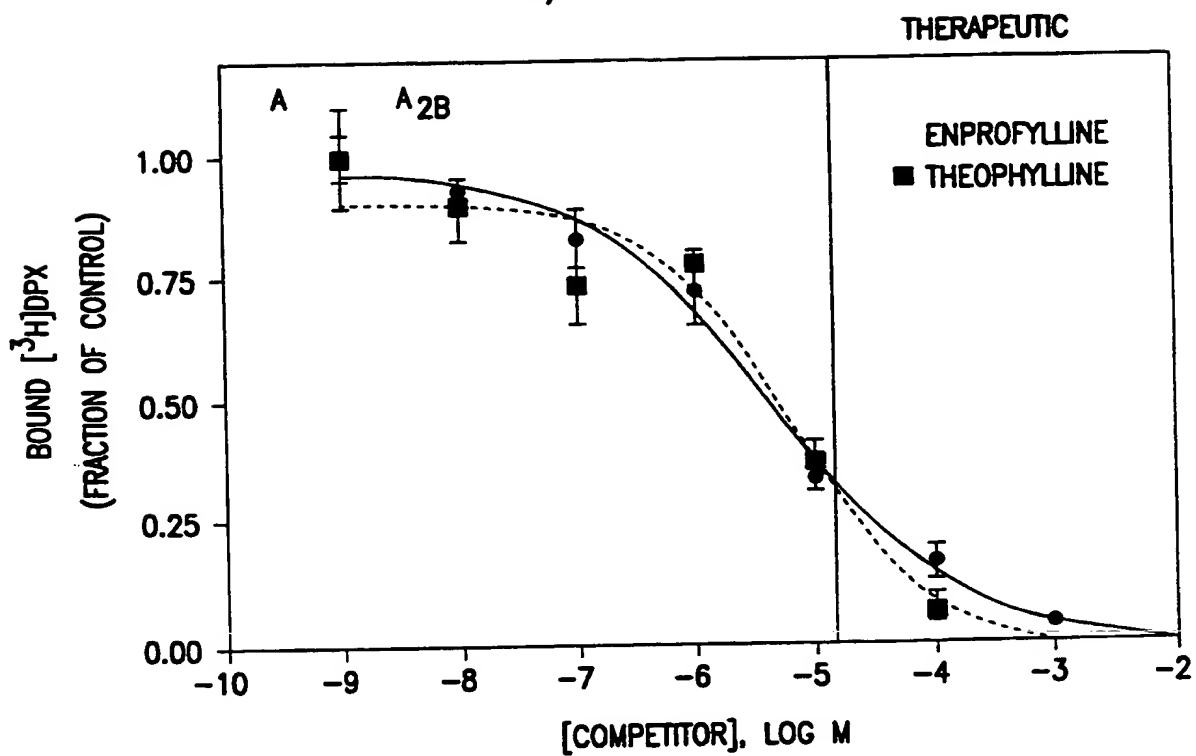


FIG. 2A

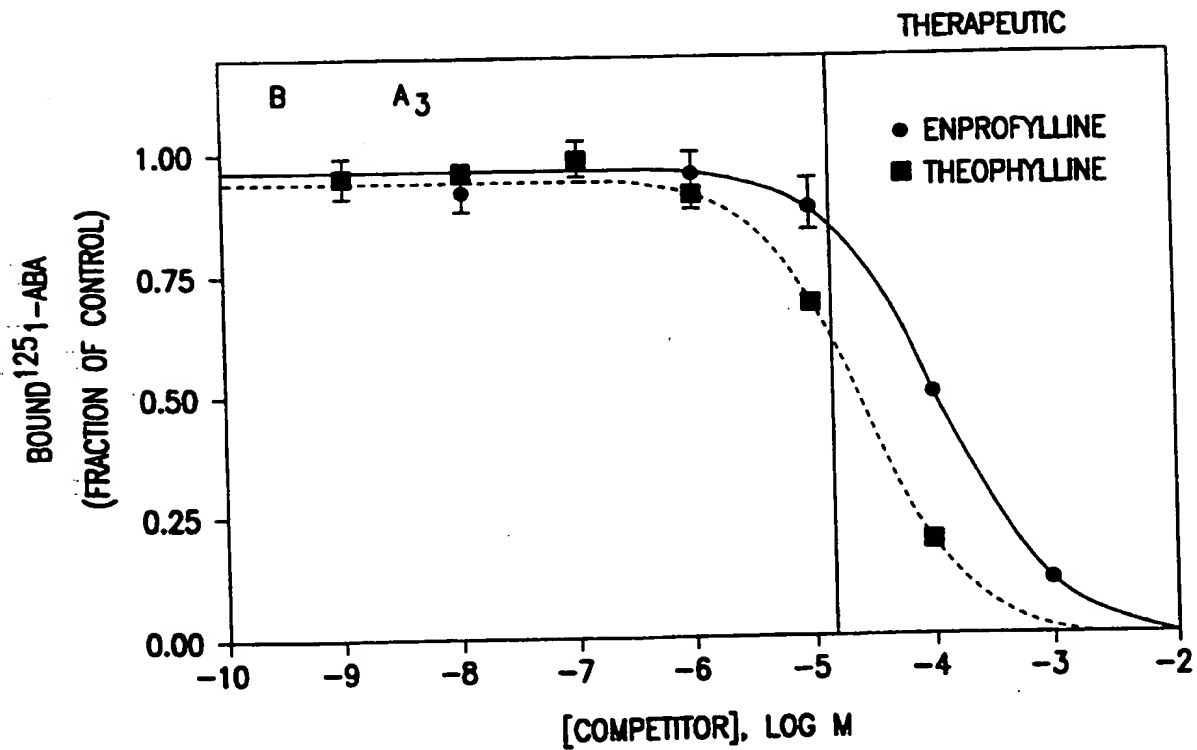


FIG. 2B

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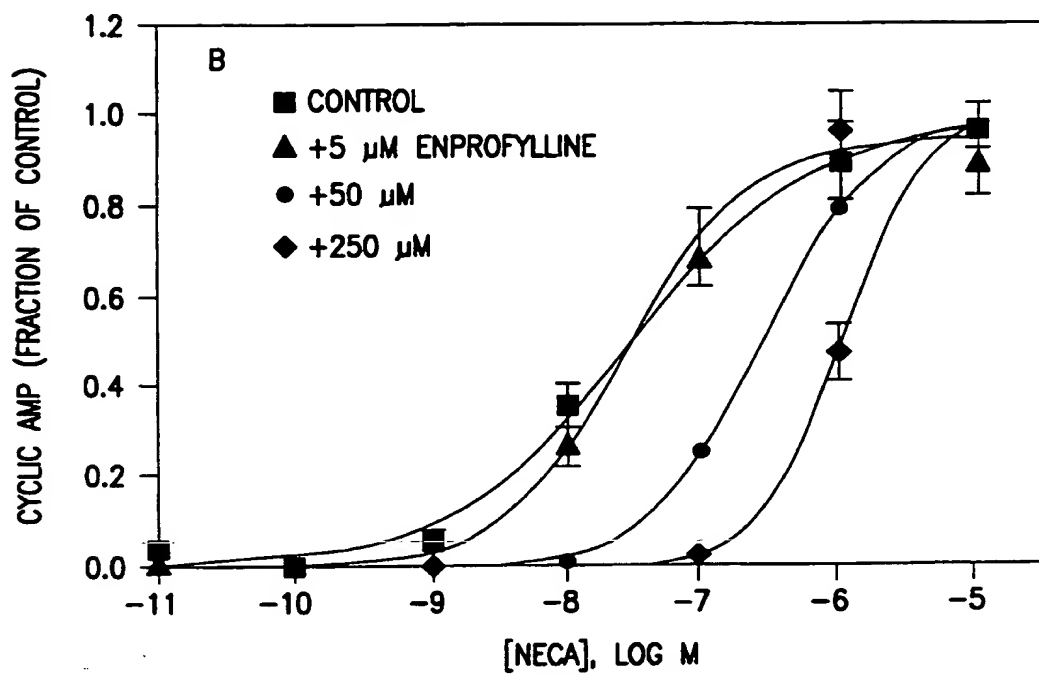


FIG. 3A

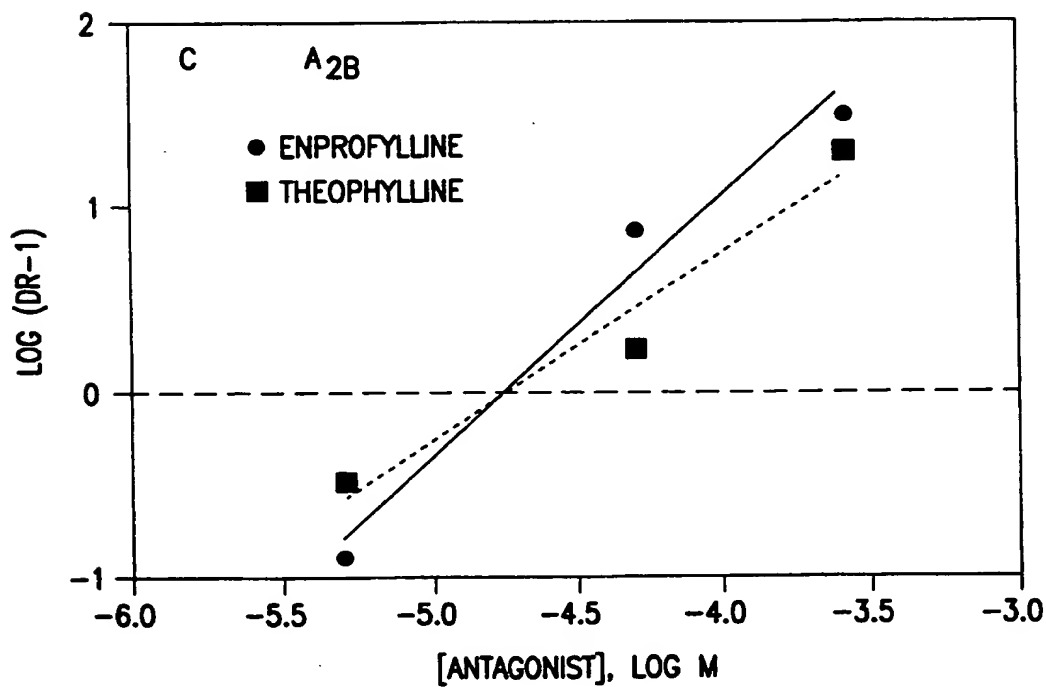


FIG. 3B

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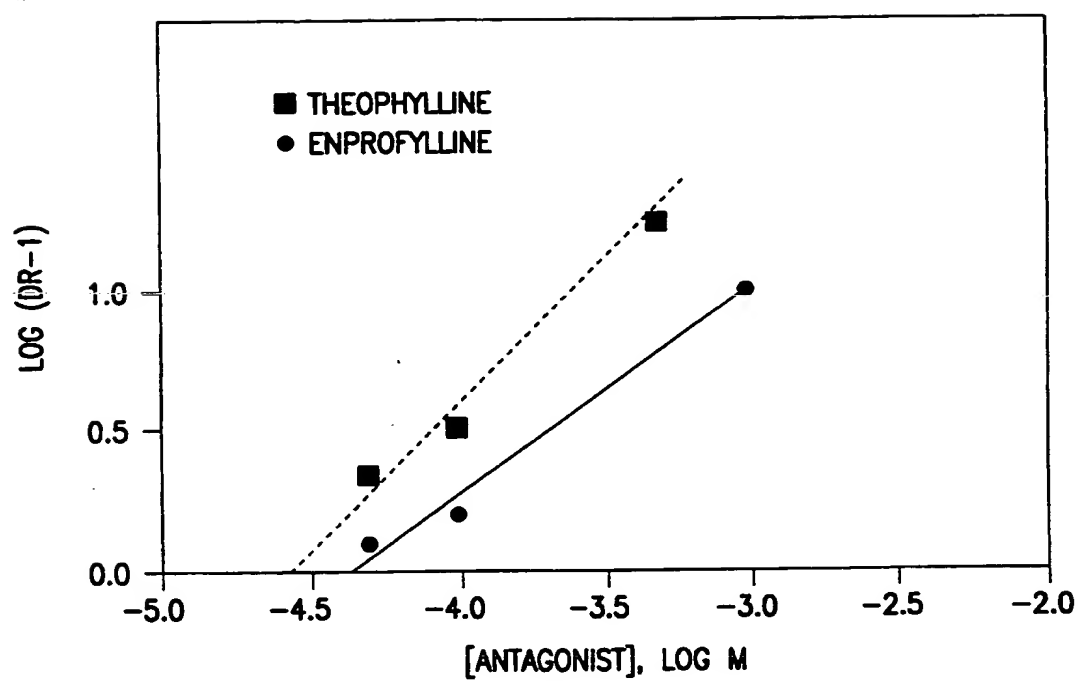


FIG. 4

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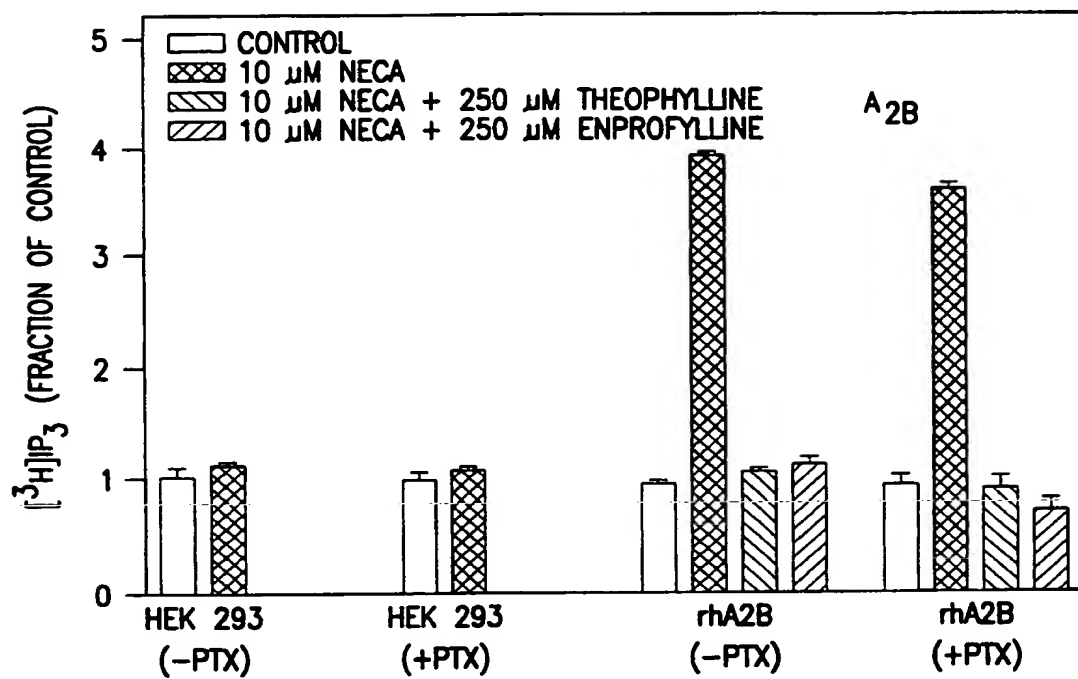


FIG. 5

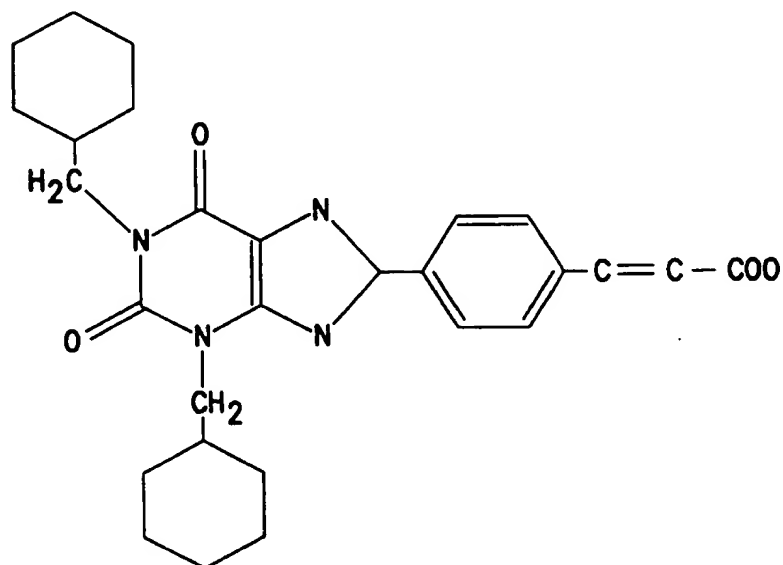


FIG. 7

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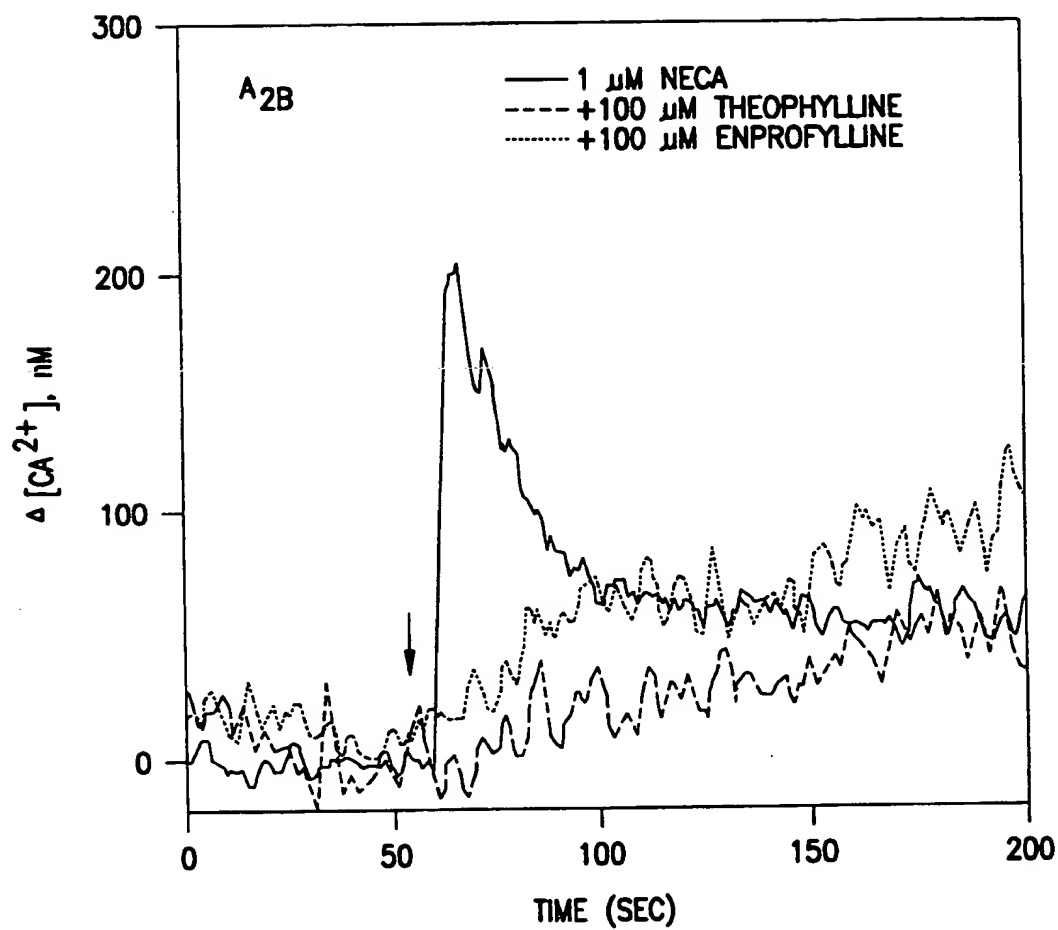


FIG. 6



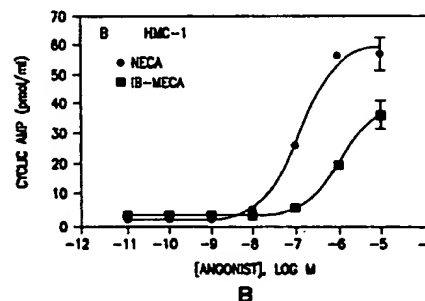
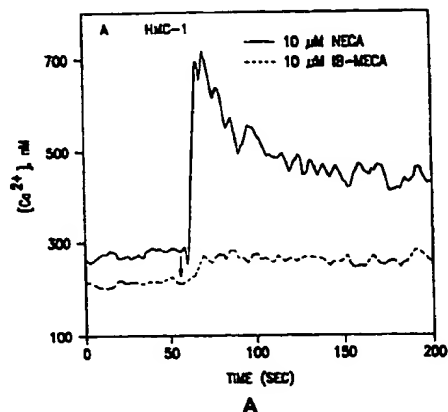
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/52	A3	(11) International Publication Number: WO 99/42093 (43) International Publication Date: 26 August 1999 (26.08.99)
(21) International Application Number: PCT/US99/04009 (22) International Filing Date: 24 February 1999 (24.02.99) (30) Priority Data: 09/027,649 24 February 1998 (24.02.98) US (71) Applicant (for all designated States except US): UNIVERSITY OF VIRGINIA [US/US]; The Rector and Visitors of the University of Virginia, Washington Hall, East Range, Charlottesville, VA 22903-2442 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): LINDEN, Joel, M. [US/US]; 207 Harvest Drive, Charlottesville, VA 22903 (US). (74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 28 October 1999 (28.10.99)

(54) Title: ANTAGONISTS OF A2B HUMAN ADENOSINE RECEPTORS

(57) Abstract

The invention concerns the use of 8-phenylxanthines, 8-cycloalkylxanthines or 8-substituted xanthine derivatives to specifically modulate the physiologic role of the A2B adenosine receptor.



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INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 99/04009

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 98 47509 A (CONNOLLY DANIEL T ; SEARLE & CO (US); SEIBERT KAREN (US); RONIKE B) 29 October 1998 (1998-10-29) abstract page 3, paragraph 2 page 5, paragraph 2; claims 1,2,5 ---	1-3,7,8, 11-13
X	WO 95 11681 A (MERCK & CO INC ; UNIV VIRGINIA (US); DOYLE MICHAEL P (US); JACOBSON) 4 May 1995 (1995-05-04) abstract page 1, line 15 - line 26 page 12, line 10 - page 14, line 4 page 19, line 21 - page 21, line 33; claims; figure 15D --- -/--	1-8, 10-13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

1 September 1999

Date of mailing of the international search report

13/09/1999

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/04009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 04280 A (WELLCOME FOUND ;DALUGE SUSAN MARY (US); WHITE HELEN LYN (US)) 15 February 1996 (1996-02-15) abstract page 2, paragraph 2 - page 5, paragraph 1; examples 1,2 page 35 - page 39; claims	1-8, 11-13
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X	WO 90 00056 A (MARION LABORATORIES INC) 11 January 1990 (1990-01-11) cited in the application the whole document	3,4,7,8
X	EP 0 203 721 A (WELLCOME FOUND) 3 December 1986 (1986-12-03) cited in the application the whole document	2-4,7,8
X	FEOKTISTOV, IGOR ET AL: "Role of adenosine in asthma" DRUG DEV. RES. (1997), VOLUME DATE 1996, 39(3/4), 333-336 , XP002113958 the whole document	1-4,7-9, 12,13
X	FEOKTISTOV, IGOR ET AL: "Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells: an enprofylline-sensitive mechanism with implications for asthma" J. CLIN. INVEST. (1995), 96(4), 1979-86 , XP002113959 cited in the application the whole document	1-4,7-9, 12,13
X	FEOKTISTOV I. ET AL: "Adenosine A(2B) receptors." PHARMACOLOGICAL REVIEWS, (1997) 49/4 (381-402). , XP002113960 the whole document	1-4,7-9, 12,13
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INTERNATIONAL SEARCH REPORT

Inter- national Application No

PCT/US 99/04009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SMITS P ET AL: "Cardiovascular effects of two xanthines and the relation to adenosine antagonism." CLINICAL PHARMACOLOGY AND THERAPEUTICS, (1989 JUN) 45 (6) 593-9. , XP002113962 the whole document	5,9,12, 13
A	----- AUCHAMPACH, JOHN A. ET AL: "Canine mast cell adenosine receptors: cloning and expression of the A3 receptor and evidence that degranulation is mediated by the A2B receptor" MOL. PHARMACOL. (1997), 52(5), 846-860 , XP002113963 cited in the application the whole document -----	1-13

INTERNATIONAL SEARCH REPORT

In ternational application No.

PCT/US 99/04009

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 04009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-8,12,13 relate to a compound defined by reference to a desirable characteristic or property, namely an adenosine A2B receptor subtype specific inhibitor (comprising a 8-substituted xanthine derivative).

Claim 2 covers all compounds having this characteristic or property and the claims 1, 3-8 cover all 8-substituted xanthine derivatives having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned in the examples and in claims 9-11.

Claims searched completely: 9-11

Claims searched incompletely: 1-8,12,13

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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